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CO_2 Laser Photothermal Effects on Rats Skin Tissues

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Abstract
Spectroscopic and histological study of CO_2 laser interaction with rats tissues were carried out. We detected for the first time the changes in optical absorption spectra of rat's tissues with selective CO_2 laser optical densities ranging from 20.64 to 34.40W/cm^2. The laser exposed tissues show higher absorbance in hemoglobin than that for the unexposed tissue. Using the same laser and same optical densities, the absorption spectra of fresh blood were measured. Much intense peaks of hemoglobin than that for the corresponding peaks for the tissue were detected. A scheme based on recent experimental findings is proposed for explanation of this novel phenomenon. The histological study shows thermal damage of blood vessels localized at the dermis layer. At high laser optical densities a vessel rupture with hemorrhage were occurred. The results indicate a pronounced modification of skin absorption properties by laser irradiation. Such an effect is due to thermally induced biophysical and biochemical processes inside the highly heterogeneous tissue structure.
Keywords: CO_2 laser, Photothermal effects, Hemoglobin absorption, Histology

Introduction

The advantages of the CO_2 laser surgery were better hemoslasis, precision of working, non-contact dissection, less instruments at the site of operation and minimum traumatization of the surrounding tissues (Paczuska *et. al.* 2014). Thermal effects on tissue and blood by using a high power density laser have received a major attention among medical applications of lasers, such as in surgery and more specific in dermatology (Niemz 2007). In most of the studies that are using high power density laser the researches are focusing on thermal therapy to attain optimum irradiation conditions (Omar *et. al.* 2009). During CO_2 laser exposure, absorption and radiationless transition convert the laser power density into heat within the front part of the tissue. Rapid localized heating causes large thermal transients and shock waves which may propagate into the blood vessels located at the dermis layer of the tissue. The experimental results demonstrate that above 60 to 70° C structural proteins including collagins are denaturated (Anderson & Parrish 1983). Selective photothermolysis for laser treatment of port wine stains was developed recently using Monte Carlo technique (Dong *et. al.* 2012). The simulation demonstrates that an optimal laser power density can cause vessel damage. Quantitative comparisons of heating effect to vessel diameter and vessel depth have been shown to be dependent on laser power densities. Up to now, the effect of thermal treatment with optical radiation on metabolic processes occurring in the body has remained insufficiently studied. (Zalesskaya *et. al.* 2011).

In the present work, we have carried out a comprehensive analysis of the changes in the absorption spectra of blood vessels inside the tissue and fresh blood withdrawn from the heart region of the animal. A mechanism based on the interaction of CO_2 laser radiation (λ= 10.6μm) with the blood was proposed for results explanation. This mechanism was confirmed by the recent experimental and theoretical prediction using infrared laser emitting near (λ= 2μm) (Zalesskaya *et. al.* 2011).

2. Equipments and methodology

Carbon dioxide laser is used as a source of radiation with output power of this laser ranged from 0 to 15 Watts. The output power of this laser was precisely measured by using Coherent power meter head connected to a digital controller. This laser is operated in TEM_{00} mode with beam diameter of 3 mm at the tissue surface. Ten experimental Sprague- Dowley male rats of ages 10 to 12 weeks and weights of 250 to 300 grams were used. The distance between the rat tissue and the laser is fixed at 10 cm. The experimental set up for CO_2 laser exposure of rats tissues is shown in Fig. (1). Histological method is carried out to make the standard slides for examination under compound microscope. A double beam spectrophotometer model UV- 2700 supplied by Shimadzu Co. was used for absorption measurements. It covers the spectral range (190-1100 nm). The depth of the thermal damage in the tissues was measured by using a demountable ocular scale mounted onto the eye piece of the optical microscope. An amount of 1μl from heparinized fresh blood was dissolved in 10 ml of NaCl isotonic solution for spectroscopic analysis.

Results and Discussion

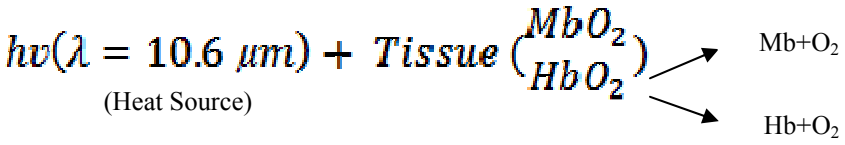
The back region of each rat skin tissue was prepared by hair removing of the exposed areas. The measured average thermal damage as a function of laser power densities (fluence) is depicted in Fig. (2), where the exposure time was fixed to 15 seconds. The average thermal damage depth at lowest laser power density (20.64

W/cm^2) is about 130 µm, increased to about 900 µm at laser power density of 34.40 W/cm^2. At these two laser power densities used to expose labeled areas at the surface of the tissue, and varying degrees of confinement of the thermal damage exists. During laser exposure, heat transfer occurs and entire labeled tissue area is heated relatively uniformly, causing nonspecific coagulation necrosis even though specific pigments are the sites of optical absorption. Optical microscopy showed a permanent damage to the blood vessels membranes occurred at the laser optical density of 20.64W/cm^2. When the laser power density increased a vessel rupture with hemorrhage was occurred as is shown in Fig. (3). This progression of effects is presumably related to vessel target temperature achieved. Sufficient transfer of heat from the absorber (oxyhemoglobin in the erythrocytes) to the vessel wall coagulates and destroys the vessels. The vessel rupture achieved at laser power density 34.40W/cm^2 is due to vaporization and subsequent shock wave damage.

The compound effects of laser thermal damage along with the laser blood vessels rupturing have shown to be appeared in hemoglobin optical properties. Fig (4) shows the absorption spectra of normal tissue along with laser exposed tissues at two different laser power densities .The peak at λ= 416nm and the two peaks situated at λ= 541nm and λ= 576nm are pertained to hemoglobin (Liu *et. al.* 2012 & Ansari & Muhajerani. 2011). The first recorded point is the thermal dissociation of oxyhemoglobin HbO_2 by the CO_2 laser irradiation forming the hemoglobin and molecular oxygen. Peaks intensities were found to increase with increasing laser power densities. These results, particularly, the deoxyhemoglobin peak at λ=416nm indicate a pronounced modification of skin absorption properties by laser irradiation. This change in absorption spectra is due to thermally induced biophysical and biochemical processes inside the highly heterogeneous tissue structure. Appearance of hemoglobin absorption in the skin is caused by damage of local chromophore during optical excitation followed by heat penetration at deeper skin layers where skin capillaries and vessels are located. The same phenomenon was observed recently by (Ferulova *et. al.* 2012) using a low power laser working in the visible region of the spectrum. They concluded that the content of skin hemoglobin has changed with changing the laser power densities. The results in Fig. (4) confirmed that the average changes in absorbance's depend on laser irradiation power densities that increases when the laser power densities increases. Conformal changes in the protein macromolecules under the influence of irradiation leads to changes in the structure of blood hemoglobin.

The most important significance of these spectroscopic changes relies on the molecular oxygen formed by the thermal dissociation of oxyhemoglobin. Formation of molecular oxygen can be considered as a key criterion in the mechanism of the therapeutic effect of infrared laser radiation. This criterion is based on restoring the molecular oxygen O_2 concentration to the levels required for normal cell metabolism. Laser induced thermal dissociation of blood HbO_2 provides achievement of this criterion by enabling the control of local oxygen concentration in the irradiated region by controlling the laser power densities. Liu *et. al.* 2012 carried out a systematic study of absorption spectra of hemoglobin at different oxygen partial pressure (PO_2) levels. They showed a specific variation of absorption spectra of Hb as PO_2 decreased from higher 100 to 0mmHg. Fig(4) indicated that the variation range of absorption spectra at 416 nm was much larger than that of the absorption spectra at 541 nm and 576 nm, indicating that the absorption spectrum at 416 nm is much more sensitive to alterations in PO_2.These results ascertain that the change in absorption ratios of Hb at λ=416nm as a function of laser power densities is due to lowering of PO_2. This decrease in the PO_2 indicating alteration in quaternary structure, which make it easier for O_2 molecule to dissociate from the Hb-subunits. The absorption ratios of Hb at λ=416nm (Fig.4) are 37.7% and 22.4% at laser power densities of 20.64 and 34.40W/cm^2 respectively. These percentage ratios determine the amount of oxygen saturation. According to hemoglobin saturation curve, these percentages give values of PO_2 equal to 21 and 15 mmHg respectively. Patients might have cyanopathy when the absorption ratio is lower than 85% (Kaur *et. al.* 2011). These spectroscopic measurements which represent hemoglobin oxygen saturation percentages accompanying with the corresponding values of PO_2 can thus be used to verify a novel partial oxygen pressure sensor usually called oximeter.

For the support of the change in hemoglobin absorption in tissues a fresh blood was withdrawn from the rat heart veins and then immediately exposed to laser irradiation. Fig. (5) shows the immediately measured absorption spectra of rat blood after expose to the same laser power densities as in the previous Fig.(4). Absorption intensities in Hb peaks are higher than that detected for the case of exposed tissue. The reasons of such difference belong to the following reasons: Firstly, in solution it is possible to control the concentration of blood hemoglobin solution which is intensity dependent, and secondly, the presence of oxymyoglobin MbO_2 in muscle tissue (Asimov *et. al.* 2012) complicate the situation, while the solution is free from Mb. The following scheme is proposed for the mechanism of interaction of CO_2 laser with the rat's tissue:



According to this scheme the decrease in the enhancement absorption intensities in relative to the fresh blood can be explained by the presence of Mb, a protein that is similar in structure to the Hb, during and after the CO_2 irradiation. Such myoglobin does not exist in directly irradiated blood. This precursor acts as scattering centers for the laser irradiation causes an increase in scattering on account of absorption. Furthermore, the release of oxygen from HbO_2 during the laser irradiation alters the structure of the Hb which results in an increase in the concentration of deoxyhemoglobin (Liu *et. al.* 2012). This fact gives a further evidence for interpreting the increase in absorbance with the laser power densities.

In the laser therapy thermal dissociation of MbO_2 in the condition of low oxygen supply can cause deep hypoxia in the muscles (Lai *et. al.* 2009). This effect must be taken into accounts in laser therapy using infrared irradiation.

Conclusions

We were able to detect for the first time the compound morphological and spectroscopic changes of rat's tissues when exposed to different CO_2 laser power densities. Based on the correlation between specific spectral changes in tissue and the PO_2 levels, it possible to estimate the PO_2 simply by measuring spectroscopic alterations in tissue at a given wavelength. This phenomenon can be used for optimizing a clinical oximeter. Thermal laser safety should be taken into consideration for avoiding any biological or photochemical effects when CO_2 laser was used in photothermal therapy.

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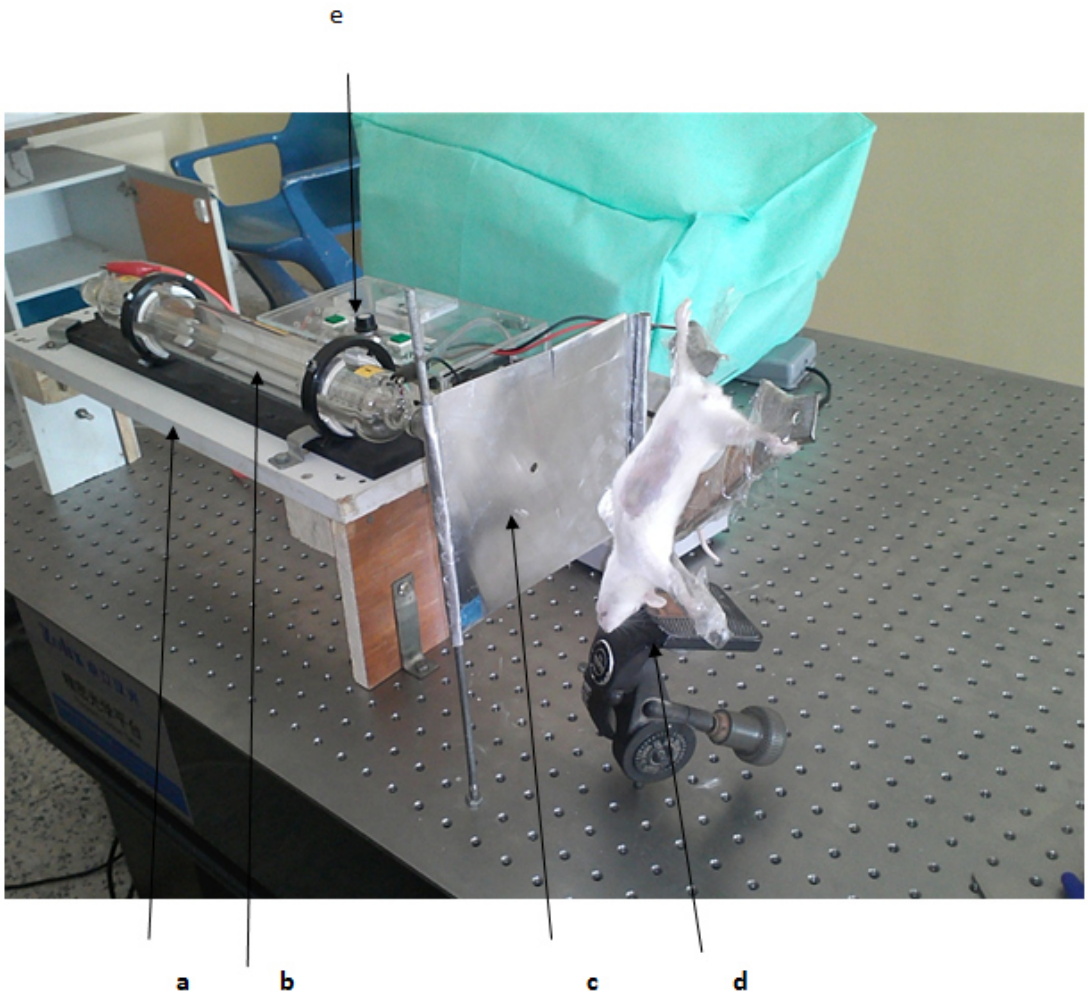


Figure 1. The experimental set-up, a- laser mount, b- CO_2 laser, c- Aluminums shutter d- Rat holder, e- power supply

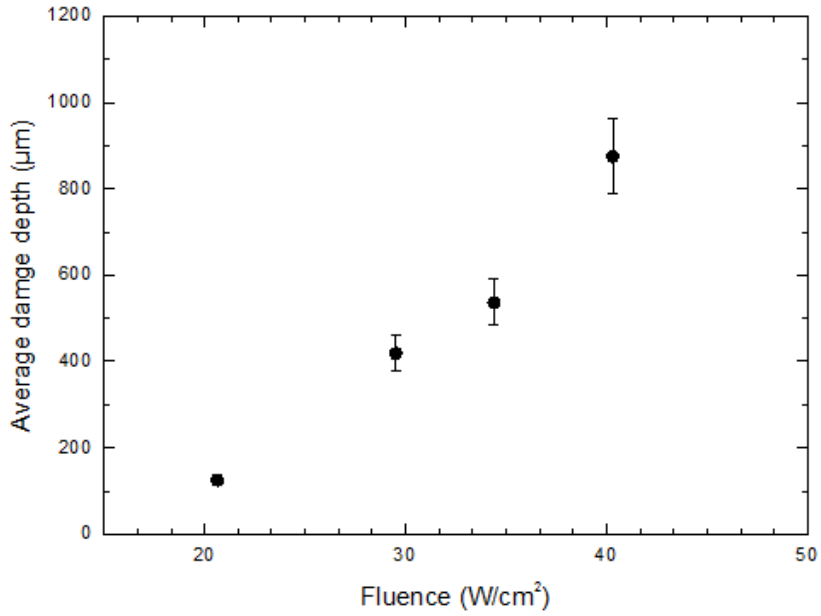
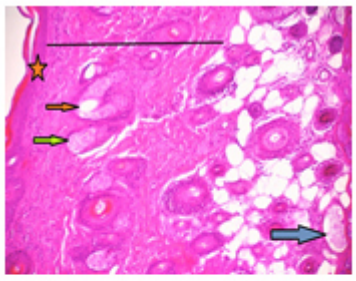
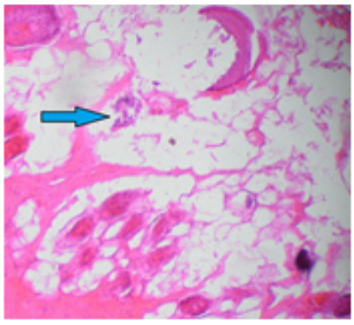


Figure 2. Average damage depth as a function of CO_2 laser fluence



a



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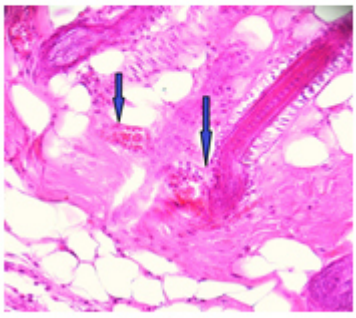

c

Figure 3. a- Control tissue, b- Exposed tissue (20.64 W/cm^2),
c- Exposed tissue (34.40 W/cm^2)

Blood vessel

Sebaceous glands

Epidermal layer

Dermis layer

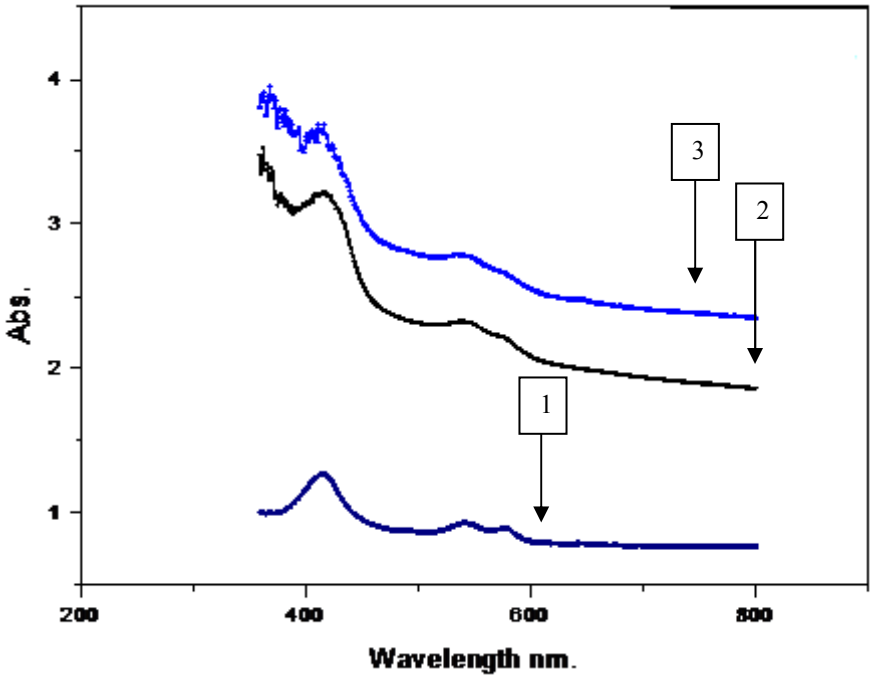

Figure 4. Absorption spectra of rat's tissue at different laser power densities.

1- control tissue, 2- tissue exposed to 20.64 W/cm^2 3- Tissue exposed to 30.40 W/cm^2

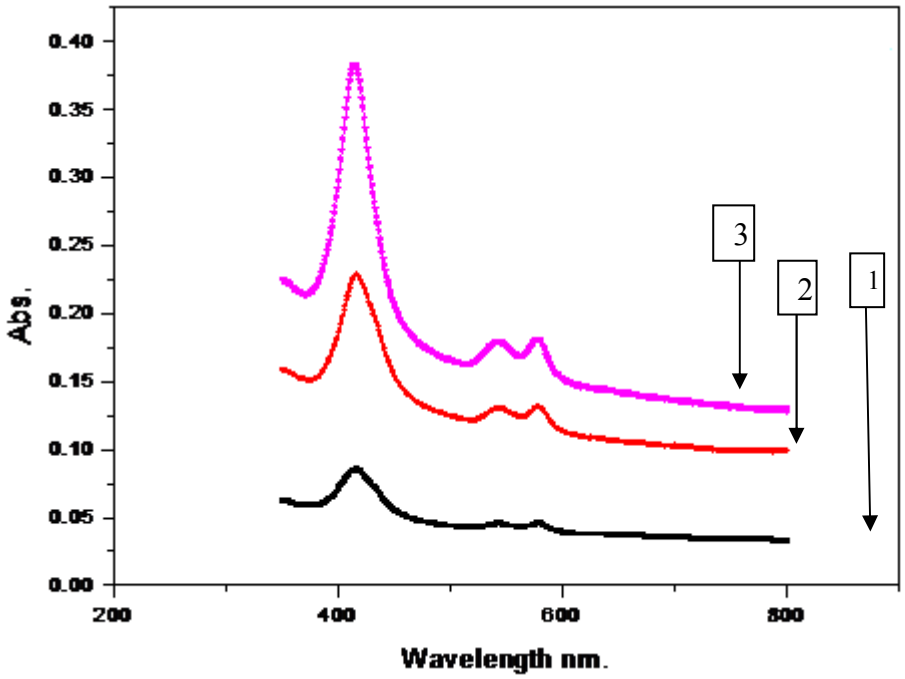

Figure 5. Absorption spectra of fresh blood withdrawn from the heart region of the rat
1- control blood, 2- blood exposed to 20.64 W/cm^2 3- blood exposed to 30.40 W/cm^2

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